

**Impact of cold storage on glucosinolate levels in seed-sprouts of
broccoli, rocket, white radish and kohlrabi**

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ABSTRACT

The effect of cold storage on glucosinolate concentration was examined in 7-day-old seed-sprouts of broccoli, kohlrabi, white radish and rocket. Principal glucosinolates identified were glucoraphanin and glucoerucin (in broccoli, kohlrabi and rocket), glucoiberin (in broccoli and kohlrabi), and glucoraphenin and glucodehydroerucin (in white radish). Generally, sprouts showed no significant changes in individual glucosinolate concentrations during storage at 4°C for 3 weeks. The exception to this was rocket, which showed a significant decline in glucoerucin and glucoraphanin after 1 and 2 weeks, respectively. These preliminary results indicate that as there is no significant loss of glucosinolates in broccoli, radish and kohlrabi sprouts, these sprouts may be stored under domestic refrigeration conditions without significant loss of potential anti-cancer compounds. Rocket sprouts, on the other hand, should be consumed soon after purchase if glucosinolate levels are to be maintained.

KEYWORDS:

brassicacae, refrigeration, isothiocyanates, cancer

INTRODUCTION

Broccoli has been identified as a vegetable with potential anti-cancer activity due to high levels of glucoraphanin, which can hydrolyse to form sulphoraphane, an isothiocyanate with high capacity to induce phase 2 enzymes. Moreover, broccoli sprouts have been reported to have 20-50 times the glucoraphanin concentration of mature broccoli heads, and consequently greater phase 2 induction capacity (Fahey et al. 1997). Apart from sulphoraphane, a number of other isothiocyanates are capable of inducing phase 2 enzymes, derived from glucosinolates in various species (Tawfiq et al. 1995; Rose et al. 2000; Fahey et al. 2001). As with broccoli, however, glucosinolate concentrations tend to be high in sprouted seed (O'Hare et al. 2007).

Commercially, seed-sprouts are sold in containers which are then kept in domestic refrigerators until used. Although glucosinolate concentrations may be high at time of purchase, there appears to be no data documenting the stability of different glucosinolates during cold storage. The present trial investigated changes in glucosinolate levels of four species of brassicaceous sprouts and the implications of domestic refrigeration on the content of bioactive glucosinolates.

MATERIALS AND METHODS

Plant Material, Sprouting Conditions and Cold Storage

Commercially available seed of broccoli (*Brassica oleracea* var. *italica*, BroccoSprout™), kohlrabi (*B. oleracea* var. *gongylodes*, 'Purple Vienna'), rocket (*Eruca sativa*, un-named variety), summer white radish (*Raphanus sativus*, 'Minowase Summer Hybrid') and winter white radish (*Raphanus sativus*, an un-named variety of winter daikon) were sanitised for 2 h in 2500 $\mu\text{L}\cdot\text{L}^{-1}$ sodium hypochlorite, rinsed with tap water and sprouted for 7 days inside a growth chamber

of a commercial hydroponic facility (24°C, 24-hour lighting and a 3-hourly watering with a commercial nutrient solution [NZ Hydroponics]). After 7 days, sprouts were cut from their root mats, divided into 8 lots weighing more than 4g each and placed in polypropylene bags (140mm x 200mm). The bags were sealed, perforated for ventilation, weighed and placed at 4°C in the dark simulating a domestic refrigerator. Bags were removed at 1, 4, 6, 7, 11(or 12), 14, 17, and 21 days for glucosinolate analysis (see below). On removal, bags were re-weighed and the atmosphere within the bag (oxygen and carbon dioxide levels) monitored using a M.A.P. Test 4000 packaging atmosphere analyser (Hitech Instruments Ltd., Luton England) to ensure the bags had not developed modified atmospheres. The entire experiment was replicated three times.

Glucosinolate extraction and analysis

Ten sprouts for radish varieties (0.15-0.22 g/sprout) and twenty sprouts for the smaller remaining species (0.02-0.04 g/sprout) were weighed and added to approximately 25ml boiling water for 6 minutes to inactivate myrosinase enzyme activity. Sprouts were homogenised with an Ultra-Turrax (IKA Labortechnik) for two minutes and then centrifuged for 15 minutes at 14000rpm. The supernatant was collected and filtered through a Whatmans No.1 filter paper. The filtrate was made up to 20ml with distilled water and refiltered through a 0.2µm syringe filter.

Supernatants were analysed for glucosinolates by HPLC-UV and HPLC-MS as described by West et al. (2002). Quantification of individual glucosinolates was initially based on commercially available high-purity (99.3%) sinigrin (Fluka) and expressed as sinigrin-equivalents. Minute amounts of more expensive standards were used to calculate the relative integrated absorbance areas for equimolar concentrations of glucoiberin, progoitrin, glucoraphanin, glucoraphenin and glucoerucin standards

(Glucosinolate.com, Denmark). Conversion factors were then used to convert sinigrin-equivalents to actual glucosinolate concentrations. In the case of glucohydroerucin where no standard was available, the conversion factor for glucoerucin was used.

RESULTS AND DISCUSSION

Of the five Brassicaceae sprout samples tested (broccoli, kohlrabi, rocket, winter white radish and summer white radish), only rocket sprouts showed a significant change in glucosinolate levels over three weeks storage at 4°C (Fig. 1A). In rocket, glucoerucin concentration declined after 1 week storage, while glucoraphanin declined after 2 weeks. Changes in glucosinolate levels in broccoli, kohlrabi, winter white radish and summer white radish sprouts were not significant (Fig. 1B-1E), despite glucoerucin and glucoraphanin being also present in both broccoli and kohlrabi (Table 1). Weight loss measurements generally were in the range of 0.2-2.1%, indicating loss of moisture was very small and therefore likely to have minimal impact on metabolism. Similarly, measurement of oxygen and carbon dioxide within bags remained in the range 20.0-20.6% and 0.3-0.8%, respectively, indicating a non-modified atmosphere and aerobic conditions.

Glucoerucin and glucoraphanin have been reported to be the major glucosinolates present in rocket sprouts (Barillari et al. 2005). The major glucosinolate, 4-mercaptobutyl glucosinolate, found in rocket leaves by Bennett et al. (2002) was not detected in sprouts in the present study or by Barillari et al (2005). Although we did not detect 4-mercaptobutyl glucosinolate in the current study, we have found it to be a major component in sprouts of at least one rocket cultivar (Yates

Australia), which would indicate that there is some variation in glucosinolate components between different rocket varieties.

Seven-day old broccoli sprouts used in this experiment contained glucoraphanin, glucoiberin and glucoerucin (Table 1), with trace amounts of sinigrin and progoitrin (data not shown). This profile is similar to that reported by West et al (2002) whose HPLC protocol was used in our experiments. The additional glucosinolates (4-hydroxyglucobassicin and glucoibervirin) present in 2-3 day old broccoli sprouts in the West study were not detected in the present 7 day old sprouts, although we have previously detected these in younger sprouts (2-3 days old) (data not shown).

Glucoraphanin was the major glucosinolate found in 7 day old broccoli sprouts, in agreement with previous reports by Fahey et al. (1997); Pereira et al. (2002) and West et al. (2002). However, glucoraphanin, glucoiberin and glucoerucin concentrations of 1.70, 0.55 and 0.35 $\mu\text{mol sinigrin equivalent}\cdot\text{g}^{-1}$ FW respectively (Table 1) were low when compared with other broccoli cultivars. Although the differences may have been due to cultivar variation, the low values in our study may have been due to lower initial glucosinolate levels in the seed. Measured glucoraphanin for the BroccoSprouts™ broccoli seed used in this study was 22.6 $\mu\text{mol}\cdot\text{g}^{-1}$ which is approximately half the level quoted for BroccoSprouts™ broccoli seed in West et al. (2004).

In the present study, winter white radish and summer white radish sprouts were found to contain principally glucoraphenin and glucodehydroerucin, although in significantly different concentrations (Table 1). Summer white radish sprouts contained twice the concentration of glucodehydroerucin and less than half the glucoraphenin content of winter white radish. Apart from indicating there may be

considerable variation in glucosinolate contents between radish cultivars, it also impacts on potential anti-cancer or anti-mutagenic activity, as the isothiocyanate derived from glucoraphenin would appear to have greater potency than that derived from glucohydroerucin (Posner et al. 1994; Nakamura et al. 2001).

The present study indicates that storage of sprouts at 4°C, as recommended for domestic refrigerators by the USDA (2005), is unlikely to have significant affect on endogenous glucosinolate concentrations of broccoli, white radish or kohlrabi sprouts. However, due to the limited number of replicates performed, these finding are tentative. By contrast, rocket sprouts exhibited significant decline in glucoraphanin and glucoerucin, both of which have been shown to have derivatives with anti-cancer potential. Consequently, if glucosinolate concentration is to be maximised, rocket sprouts should be consumed soon after purchase.

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Table 1 - Average glucosinolate contents (\pm SE) of 7-day-old seed-sprouts stored at 4°C. Values are also expressed as ‘sinigrin-equivalents’ for comparison with other studies (GR, glucoraphanin; GE, glucoerucin; GI, glucoiberin; GRe, glucoraphenin; GDE, glucodehydroerucin).

| | Glucosinolate concentration | | | | | | | |
|-----|---|------------------------|-------------------------|-------------------------|--|------------------------|------------------------|-------------------------|
| | $\mu\text{mol}\cdot\text{g}^{-1}$ FW actual glucosinolate | | | | $\mu\text{mol}\cdot\text{g}^{-1}$ FW sinigrin equivalent | | | |
| | broccoli | kohl rabi | winter radish | summer radish | broccoli | kohl rabi | winter radish | summer radish |
| GR | 5.55 (± 1.53) | 6.36 (± 1.67) | | | 1.70 (± 0.47) | 1.95 (± 0.51) | | |
| GE | 0.53 (± 0.38) | 0.72 (± 0.41) | | | 0.35 (± 0.25) | 0.47 (± 0.27) | | |
| GI | 1.19 (± 0.27) | 3.98 (± 1.93) | | | 0.55 (± 0.12) | 1.83 (± 0.89) | | |
| GRe | | | 11.44 (± 2.62) | 3.34 (± 1.53) | | | 3.55 (± 0.81) | 1.04 (± 0.47) |
| GDE | | | 6.89 (± 1.81) | 16.56 (± 3.98) | | | 4.53 (± 1.19) | 10.88 (± 2.62) |

